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Renal Progenitors Derived from Urine for Personalized Diagnosis of Kidney Diseases

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Abstract
Background: Chronic kidney disease affects 10% of the world population, and it is associated with progression to end-stage kidney disease and increased morbidity and mortality. The advent of multi-omics technologies has expanded our knowledge on the complexity of kidney diseases, revealing their frequent genetic etiology, particularly in children and young subjects. Genetic heterogeneity and drug screening require patient-derived disease models to establish a correct diagnosis and evaluate new potential treatments and outcomes. ***Summary:*** Patient-derived renal progenitors can be isolated from urine to set up proper disease modeling. This strategy allows to make diagnosis of genetic kidney disease in patients carrying unknown significance variants or uncover variants missed from peripheral blood analysis. Furthermore, urinary-derived tubuloids obtained from renal progenitors of patients appear to be potentially valuable for modeling kidney diseases to test ex vivo treatment efficacy or to develop new therapeutic approaches. Finally, renal progenitors derived from urine can provide insights into acute kidney injury and predict kidney function recovery and outcome. ***Key Messages:*** Renal progenitors derived from urine are a promising new noninvasive and easy-to-handle tool, which improves the rate of diagnosis and the therapeutic choice, paving the way toward a personalized healthcare.

Introduction

Chronic kidney disease (CKD) affects more than 10% of the population, and it is associated with progression towards end stage kidney disease [1]. The high number of affected individuals and the significant adverse impact of CKD should prompt enhanced efforts for a better diagnosis of kidney diseases and treatment. The past two decades have witnessed a revolution in understanding the pathogenic mechanisms underpinning kidney diseases, mainly due to the development of multi-omics technologies. The huge amount of retrieved patient information provides the basis for deciphering complex diseases, highlighting relevant clues related to the cause of disease, the risk of progression, and the resistance to specific treatments, profiling a personalized signature [2]. Although these results are extremely useful in dissecting

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the heterogeneity of kidney diseases, they are often not conclusive. Therefore, patient-derived disease models that recapitulate crucial mechanisms underlying kidney disease are more important than ever. A desirable disease model should be obtained by noninvasive cell isolation methods followed by simple culture techniques. The kidney, unlike other organs, gives the possibility to use the urine sample as an unlimited source of patient-derived kidney cells that can be harvested noninvasively using a cost-effective and simple method of isolation. It is well known that exfoliated kidney cells in urine represent a potentially useful source to noninvasively diagnose kidney diseases and prognosticate their progression [3, 4]. The main types of kidney cells voided in urine are podocytes and proximal tubular cells [4]. These cells exhibit a limited expandability and lifespan due to their mature differentiation state, requiring immortalization to maintain them in vitro [5–10]. Immortalized urinary podocytes and tubular cells of healthy volunteers and patients established by hTERT, temperature-sensitive SV40 T antigen transfection or the introduction of viral oncogenes possess strong proliferative potential and could acquire the phenotype of fully differentiated cells in response to changes in culture conditions (such as temperature, exposure to retinoic acid, retinoic acid + vitamin D3, hydrocortisone + EGF + tri-iodothyronine) [9, 11–15]. Even if several studies highlight the usefulness of these immortalized-kidney cells for disease modeling [6, 12, 16, 17], drug screening [12, 14], and bioengineering application [13, 18], the invasive procedure of immortalization could affect the transcriptome and interfere with cellular pathways of cells, which might lose their sensitivity to external stimuli [5, 19]. Therefore, cell lines become a *faux* representation of biological system and make it unreliable and with a limited use in practice [5, 19]. Recently, urine-derived kidney cells have been reprogrammed to a pluripotent state so-called induced pluripotent stem cells (iPSCs) [20, 21]. When these cells are properly stimulated, they give rise to kidney organoids, in vitro complex renal structures that closely mimic in vivo architecture of nephrons [22–25]. The iPSC-derived kidney organoids have several limitations, including short lifespan, low reproducibility, off-target cell populations, incomplete cellular complexity, and immature structures [26]. The unsolved challenges of this technology along with the need of having high expertise, make it not affordable from the majority of laboratories for diagnostic purposes. Therefore, the emergence of adult stem cell cultures has received widespread attention as having the potential to be easily isolated from urine samples of patients [8, 27–29]. Specifically, isolation of a population of adult renal progenitor cells (RPC) from urine of patients with bipotent capacity driving podocyte and tubular differentiation has paved the way to study these cells for regenerative purposes. Indeed, several evidences have proved that administration of u-RPC in rodent models of acute kidney injury (AKI) and CKD [30–32] reduced the kidney injury by different mechanisms such as engraftment and differentiation into kidney parenchymal cells [30], exosome-mediated secretion of factors [33], anti-oxidative [32], anti-fibrotic [32], pro-proliferative [30] and antiapoptotic effects [30, 31]. Moreover, u-RPC exhibited also anti-inflammatory and immunomodulatory effects, reducing peripheral blood mononuclear cell apoptosis, inhibiting lymphocyte proliferation, increasing T regulatory cells, and reducing T helper 1 cells [34].

More importantly, isolation of RPC from the urine of patients has paved the way to build up personalized disease models [35, 36]. Growing evidence supports patient-derived renal progenitors as a new noninvasive, powerful, and easy-to-handle tool amenable to reproducing patient kidney disease in vitro. Thus, this review will focus on the remarkable aspects of this tool as an outstanding personalized diagnostic strategy to allow the access of patients to personalized care models.

Urine Renal Progenitors for Patient-Derived Cell-Based Models

A major challenge for experimental research on kidney disease has been the identification of a biologically relevant kidney cell type for this purpose. In adult human kidney, a population of RPC is interspersed within the nephron epithelium, among parietal epithelial cells of Bowman's capsule, and in tubular segments, with a higher frequency in the S3 segment of the proximal tubule [37–39]. So far, several markers have been used to identify this population in human and mouse kidney (CD133, CD24, CD106, PAX2, PAX8, SIX2, SOX9) along with a self-renewing potential and differentiation ability forming new podocytes and tubular cells during regenerative processes occurring in glomerular and tubular diseases [37–44]. In the last decade, many evidence demonstrate that their loss in urine can occur under both physiological and pathological conditions in adults, children, and preterm neonates, with an increased excretion rate in proteinuric conditions [8, 35, 36]. Specifically, they demonstrated that RPC are the only urinary cells that can undergo numerous rounds of cell division in culture, exhibiting clonogenic and amplification potential over

time [36]. Transcriptomic profile analysis proved that urine-derived RPC (u-RPC) are the same as those residing in the kidney in terms of phenotypic and functional markers [36, 45]. More recently, single-cell RNA sequencing (scRNAseq) analysis confirmed the presence of a population of RPC in urine samples endowed with bipotency, giving rise potentially to both podocytes and tubular cells [46]. In agreement with this, it is not rare to isolate u-RPC-expressing stem cell markers (i.e., CD24 and CD133) along with podocyte- or tubular-specific markers, showing several grades of commitment [35, 36]. Indeed, whereas u-RPC are properly stimulated according to specific protocols, they easily acquire the mature phenotype and the functional properties of either podocytes (retinoic acid treatment for 2 days) or tubular cells (hepatocyte growth factor treatment for 21 days) [35, 36]. Remarkably, this evidence demonstrates that RPC are the only urinary cell population that can be easily amplified long-term in culture and exhibit a commitment to more mature podocyte or tubular stage. Both these features make cellular cultures of u-RPC suitable to interrogate patient-specific biology and to create a biobank of patient-derived RPC. Thus, u-RPC are a promising tool for future wide-ranging applications in personalized medicine.

Urine Renal Progenitors for Diagnosis of Genetic Kidney Diseases

Establishing a patient genetic profile is a prerequisite for understanding disease pathogenic mechanisms and developing personalized medical treatments [47]. Patient-derived RPC that retains the genetic signature of the disease is an excellent tool to reproduce in vitro the complexity of the pathophysiological aspects of the disorder. Accordingly, u-RPC have been used to establish cellular models of genetic kidney diseases involving podocytes or tubular cells [5, 11, 36, 48–50]. U-RPC have found their first application to investigate the pathogenic role of variants of unknown clinical significance in pediatric patients with steroid-resistant nephrotic syndrome. Specifically, whereas in a patient carrying a heterozygous variant in *LMX1B* (transcriptional regulator of podocin), podocin appeared mislocalized and the cell structure was abnormal [36, 51], in a patient carrying a homozygous variant in *NPHS1*, nephrin erroneously localized in the cytoplasm [48]. As expected, alterations in slit protein assembly of podocin and nephrin at cell membrane level resulted in a severe impairment of cytoskeletal organization and in an increased podocyte detachment and mortality [36, 48]. Thus, u-RPC cultures resulted as a suitable tool for establishing the pathogenicity of variants with unknown clinical significance, demonstrating the functional role of variants in preserving podocyte structure and function. Accordingly, adoption of this strategy with diagnostic purposes can predict the risk of disease progression and allow a more aware choice for therapy in an individualized manner. A newer application of u-RPC was adopted to define the pathogenic role of intronic variants with uncertain significance in collagen genes (*COL4A3*, *COL4A4*, and *COL4A5*) in patients with clinical diagnosis of Alport syndrome [49]. On the contrary to blood cells, kidney cells are the only ones that express all collagen genes. Therefore, podocyte derived from the differentiation of u-RPC resulted as the more suited source of kidney cells to investigate the role of intronic variants on splicing pattern [49]. The impact of these alternative forms of splicing on collagen protein structures defined the genetic cause for Alport syndrome in these patients, predicting better their risk of disease progression [49]. Interestingly, u-RPC cultures were also applied for investigating the presence of a cryptic mosaicism, which is often overlooked from blood analysis [52]. The mother of a patient carrying a pathogenic variant in *COL4A5* was investigated to determine whether she could be the right donor for transplanting the kidney to the son [52]. Although the mother had microhematuria from the age of 10, she was negative on a peripheral blood test for son's variant, supporting the hypothesis of a son's de novo variant. Nevertheless, to exclude the presence of cryptic mosaicism, missed from peripheral blood analysis, mother's u-RPC cells were isolated. Unexpectedly, podocytes derived from u-RPC differentiation showed the son's variant in heterozygous, demonstrating the presence of maternal mosaicism [52]. This implied a new assessment of the risk of progression of mother disease and a reassessment of her eligibility as kidney donor. These results suggest that patient-derived u-RPC can establish the pathogenicity of unknown significance variants and uncover variants missed from peripheral blood analysis (shown in Fig. 1). Likewise, we can speculate that u-RPC may be applied to other inherited disorders of the kidney for whom immortalized cells or iPSC are currently used.

Urine Renal Progenitors for Drug Screening

To extend our knowledge on drug treatment beneficial effects and bring the therapy one step closer to personalized clinical application, patient-derived disease models

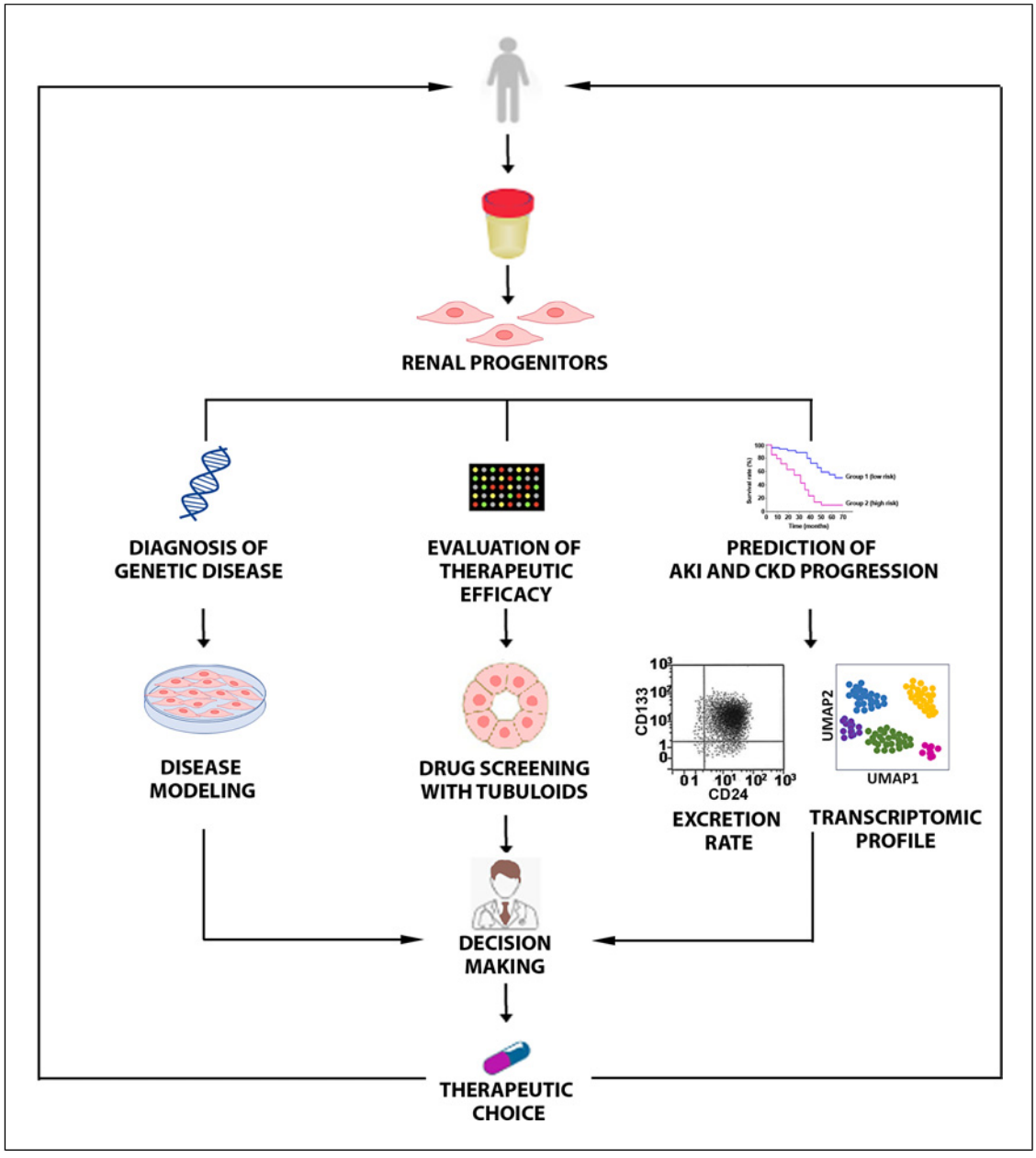

Fig. 1. Current applications of u-RPC to decipher kidney diseases and improve patient management. After urine collection from disease-affected individuals, u-RPC are cultured and used: (1) to build up patient-derived disease models to diagnose genetic kidney diseases; or (2) to generate patient-derived tubuloids to test the treatment efficacy or to develop new therapeutic approaches; or (3) to obtain insight into cellular processes underlying AKI and its progression to CKD. All these technological strategies improve the clinical decision-making and the therapeutic choice in a personalized healthcare.

appear to be promising. In particular, 3D disease models have been employed to better evaluate drug therapy efficacy and identify any potential risks associated with exposure to new chemicals and pharmaceuticals through high-throughput screening in a personalized manner [23, 53]. Renal adult stem cells can give rise to cystic, highly polarized epithelial structures termed tubuloids [54, 55]. The latter retain the characteristics of primary, functional renal epithelial cells representing distinct nephron segments, most notably of the proximal tubule [54, 56]. In contrast to iPSC-derived organoids, tubuloids can be expanded for many passages while remaining genetically

stable [55], they can be cloned at any stage of their culture and are ideally suited for CRISPR-based genomic modification [57]. Recently, CD24+ RPC from the human kidney have emerged as the cellular source for efficiently obtaining tubuloids [56]. These are an advanced in vitro model successfully used to model genetic kidney diseases and screen for therapeutic efficacy [54]. Indeed, by targeting either the *PKD1* or *PKD2* gene locus, RPC-derived tubuloids resemble cyst formation, providing an in vitro platform for modeling the human autosomal dominant polycystic kidney disease and screening treatment efficacy [56]. Tolvaptan, which is currently used to reduce cyst growth and disease progression in clinical practice [58], antagonized vasopressin effect and reduced cAMP levels mediated by antagonized vasopressin 2 receptor in PKD1−/− and PKD2−/− RPC-derived tubuloids [56]. Besides this, a step forward to a personalized drug screening was made when tubuloids were obtained from urine of patients with hereditary diseases [54, 59, 60]. Urinary-derived tubuloids obtained from a patient with cystic fibrosis were used to test the patient's response on the efficacy of the CFTR enhancer drug VX-770 [54]. Remarkably, urinary-derived tubuloids obtained from RPC of patients with cystinosis were employed for screening new treatments in order to improve the management of these patients [59]. So far, cysteamine treatment slows down the disease progression but does not correct the established renal proximal tubulopathy in patients with cystinosis. Drug screening on urinary-derived tubuloids obtained from RPC of patients with cystinosis revealed a bicalutamide-cysteamine combination treatment as a novel dual-target pharmacological approach for reducing cystine levels and for the phenotypic correction of cystinotic kidney proximal tubule cells [59]. Also, gene addition approach with wild-type cystinosin in u-RPC resulted in significant reduction in cystine levels, indicating gene-corrected u-RPC as further tool for therapy investigations [11]. Overall, these promising results pave the way to consider urinary-derived tubuloids obtained from RPC potentially valuable for modeling a wide spectrum of kidney diseases to test ex vivo treatment efficacy or to develop new therapeutic approaches for personalized purposes (shown in Fig. 1).

Urine Renal Progenitors to Explore AKI Recovery and Its Outcome

AKI occurs due to various conditions such as sepsis, hypoxia, trauma, and exposure to toxins [61]. During an episode of AKI, it is unpredictable whether a patient will recover or develop persistent kidney failure. After an AKI episode, kidney function recovery relies upon two main adaptive response programs of the kidney tubule. On the one hand, RPC self-renew and differentiate to replace lost tubular cells, regenerating at least in part the tubule segments. On the other hand, tubular cells endoreplicate and become polyploid, leading to tubular cell hypertrophy, which is associated with CKD development [38]. Therefore, a major goal of AKI research should be to identify cellular targets predicting AKI recovery or its outcome to potentially use them to prevent CKD [62]. Kidney cells in urine samples of AKI patients potentially reflect type and severity of kidney damage and provide noninvasive insights into injury and healing processes [62, 63]. scRNAseq technology has shed light on the role of kidney cells with different injured and adaptive states in AKI and CKD development [63–67]. Recently, scRNAseq analysis identified RPC in urine of AKI patients, which were characterized by an adaptive state, resembling "repair" ones in line with a regenerative signature [63]. Thus, single-cell transcriptomic of u-RPC might provide insight into cellular processes underlying AKI and its progressive outcome. So far, few studies have explicitly explored the utility of RPC in human urine for the early diagnosis and risk stratification of CKD [4]. Recent studies have shown that excretion rate of RPC in urine of patients with kidney transplanted AKI and with stage 3 non-transplanted AKI indicated kidney function recovery [68–70]. The increased number of RPC in urine might indicate an attempt of tissue regeneration, which may turn out to be an unsuccessful process resulting in further CKD progression [68–70]. Overall, RPC in urine might provide insight into the severity of kidney damage and regenerative processes after AKI, opening novel opportunities for predicting kidney function recovery and the risk of progression to CKD (shown in Fig. 1).

Urine Renal Progenitors for Personalized Medicine

An early correct diagnosis through a personalized approach can reduce morbidity and the risk of inappropriate treatments. This severely impacts on quality of life, progression to kidney failure, and related high costs for healthcare system. Developing u-RPC cultures or tubuloids from the urine of patients for deciphering the causes of genetic kidney diseases represents a significant technological innovation for a better patient stratification, leading to improved clinical decision-making and outcome prediction. In the last years, a multidisciplinary team of nephrologists, geneticians, bioinformatics, and basic

researchers developed an integrated diagnostic algorithm based on advanced genetic testing, reverse phenotyping, and personalized disease models by using cultures of u-RPC [36, 47, 71]. This approach doubles the current diagnostic rate and permits an appropriate therapeutic choice, avoiding unnecessary and potentially harmful treatments and reducing costs [71]. Additionally, recent evidence in understanding cellular processes underlying AKI and its progression to CKD suggest the possibility to screen AKI patients with a personalized diagnostic purpose. Analysis of urinary kidney cells, specifically RPC, provides insight into regenerative processes after AKI and allows patient subcategorization based on severity of damage and the risk of progression to CKD. Accordingly, the management of AKI patients might significantly benefit from this approach in terms of novel opportunities for monitoring the disease course and identifying new therapeutic targets [63]. However, the lack of specific expertise for the management of u-RPC as an exquisite diagnostic tool in most nephrology units precludes the current access of patients to this personalized strategy. Spreading the access to personalized healthcare would lead to cost saving, allocating in a more rational way the available resources [72–74]. Overall, this approach would have the potential to implement the highest quality in the management of patients with kidney diseases worldwide.

Conclusion

The advent of multi-omics technologies has led to the recognition of kidney diseases as extremely complex entities for which the etiology is only apparently known, resulting in a lack of correct diagnosis for many patients. Therefore, there is an urgent need for more efficient tools to decipher and manage the complexity of kidney diseases. Personalized disease models that recapitulate human kidney disorders appear as the most feasible ones, bridging the gap between basic and clinical research. Patient-derived u-RPC result as exquisite, easy, and ready to use noninvasive diagnostic tool and therapeutic target, desirably affordable by the majority of nephrology units. Hence, patient-derived u-RPC are revealed as a novelty to facilitate the move towards a new era of personalized healthcare in nephrology. Remarkably, patient-derived disease models might show several benefits for patients, addressing them to a correct diagnosis and therapeutic treatment. Moreover, this strategy might reduce diagnostic costs, optimizing economic resources and reasonably implementing the existing or newly developed best practices.

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Conflict of Interest Statement

No conflicts of interest, financial or otherwise, are declared by the authors.

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Author Contributions

E.L., B.M., and M.E.M. critically revised the literature, edited the manuscript, and prepared the figure. E.L. B.M., M.E.M., P.R., and L.L. revised the manuscript. All authors read and approved the final manuscript.

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